

Relative Value of Solvent and Expeller Soybean Meal for Lactating Dairy Cows¹

GLEN A. BRODERICK

US Dairy Forage Research Center
US Department of Agricultural
Agricultural Research Service
University of Wisconsin
1925 Linden Drive West
Madison 53706

ABSTRACT

Nitrogen solubility and enzymatic and rumen *in vitro* degradabilities indicated protein from expeller soybean meal was more resistant to ruminal degradation than that from solvent soybean meal. This was confirmed in Trial 1 by reduced rumen ammonia and branched-chain volatile fatty acids, and by 64% more supplemental protein escaping the rumen when cows were fed expeller soybean meal. In Trial 2, rations supplemented with either solvent or expeller soybean meal, averaging 16.4% protein, were fed to 12 cows in a crossover study. Production averaged 35.3 kg/d but was not influenced by diet. A small but significant improvement in milk to feed ratio occurred with expeller soybean meal. In Trial 3, four sources of protein were fed to 20 cows in a 4 × 4 Latin square: 6.3% solvent, 4.1% expeller (plus .3% urea), 10.0% solvent, or 6.6% expeller soybean meal. Production of milk and milk components was similar on the diets containing 6.3 and 6.6% soybean meal, intermediate on 10.0% solvent, and least on the expeller-urea diet. Milk to feed was equal and greatest on diets containing 6.6% expeller and 10.0% solvent soybean meal, indicating comparable utilization of the expeller diet containing only 60% as much supplemental protein.

INTRODUCTION

There is substantial evidence that lactating dairy cows respond with increased production to increased supply of amino acids to the small intestine. Milk protein secretion was elevated with abomasal infusion of protein (10). Using literature data, Roffler et al. (27) modeled milk production response to supplemental protein from soybean meal (SBM). Production was found to increase curvilinearly with large responses at low dietary protein but progressively decreasing response with greater supplementation. Most proteins commonly fed to dairy cows in the North Central Region of the United States, including that from alfalfa forages and SBM, are highly degradable (28). This suggests that improvement in the ruminal escape characteristics of SBM could be used to advantage, giving increased production or productive efficiency.

Expeller-processing of cottonseed meal reduces ruminal degradation (8). Heating cottonseed meal to give N solubility similar to that of expeller cottonseed meal improved N retention in lambs (29). Heat treatment of soybeans and SBM has been reported to improve N utilization in lactating cows (24).

The purpose of these studies was to compare the relative protein value for lactating cows of conventional solvent-extracted SBM and a commercial expeller SBM, which is heated to a maximum of 163°C during processing.

MATERIALS AND METHODS

Source of Soybean Meals

Expeller SBM was obtained in three batches (one for each feeding study) from West Central Cooperative (Ralston, IA 51459). This meal was prepared by a process that reaches a maximum temperature of 163°C (Dennis Stucker, personal communication). Solvent

Received February 3, 1986.

¹Mention of commercial products in this paper does not constitute endorsement by the US Department of Agriculture or the Agricultural Research Service.

SBM was from local commercial sources in Madison, WI and was obtained in three batches. Subsamples from each batch were analyzed for dry matter (DM) and crude protein (2). These samples were analyzed for total N soluble in McDougall's buffer (14), 10% (vol/vol) Burroughs' buffer (14, 33) and ficin protease (25), all of which have been correlated to ruminal degradability. Nonprotein nitrogen (NPN) was estimated as the N soluble in 5% trichloroacetic acid [TCA; (14)]. Residual N insoluble after treatment with *Streptomyces griseus* protease was determined as an alternative estimate of unavailable N (20). Each sample of SBM was also assayed for fractional rate of protein degradation by a rumen in vitro system and the proportion potentially escaping the rumen was estimated (6, 7). Mean results of these assays are summarized in Table 1.

Trial 1

Six Holstein cows (two nonlactating and four milking an average of 21.0 kg/d) equipped with ruminal cannulae and weighing an average of 698 kg were randomly assigned to three dietary treatments in a 3 × 3 Latin square.

Diets consisted of corn silage plus a corn-based concentrate mix and differed in the source of supplemental N: urea (diet U), solvent SBM (diet S-1), or expeller SBM (diet E-1) (Table 2). Diet U was also supplemented with sodium sulfate. Corn silage contained 7.3% acid detergent insoluble N (ADIN, % of total N). After a 1 wk adaptation in which all cows were fed corn silage plus the concentrate mix from S-1, diets were fed in a 3 × 3 Latin square design with 2-wk periods. Milk production data are not reported because of low production and because not all cows were lactating. Cows were weighed on 3 consecutive d at the start of the trial and at the end of each period.

Diets were fed four times daily at 6-h intervals in approximately equal proportions; concentrate and silage were not mixed prior to feeding. A weekly sample of each concentrate and the corn silage was taken and stored frozen (−20°C) until analyzed. Feed refusals were also determined daily and feed offered adjusted to yield weighbacks of less than 5% of amount fed. The actual proportion of dietary DM from each component was computed from DM determined by toluene distillation (15) and at 105°C (2) for silage and concentrates, respectively. Diet ingredients were also analyzed

TABLE 1. Solubility and in vitro degradability of protein from solvent and expeller soybean meals.¹

Item	Solvent SBM		Expeller SBM	
	\bar{X}	SE	\bar{X}	SE
Crude protein, %	46.15	1.23	44.74	.45
N Solubility, % ² (McDougall's buffer)	27.22	2.85	6.44	.44
N Solubility, % ² (10% vol/vol Burroughs' buffer)	27.26	3.36	5.96	1.83
N Solubility, % ³ (Ficin)	77.91	1.36	70.33	.76
NPN, % ⁴ (5% wt/vol TCA)	5.08	1.54	5.38	1.19
Residual N, % ⁵ (<i>Streptococcus griseus</i>)	.69	.30	.54	.14
In vitro N degradation (k_d), h ^{−1}	.095	.010	.034	.004
Estimated escape, % ⁶	39	...	64	...

¹ Means and standard errors from samples of each batch of solvent and expeller soybean meal (SBM).

² Proportion of total nitrogen soluble in McDougall's (14) and Burroughs' (14, 33) buffers.

³ Proportion of total nitrogen solubilized by treatment for 4 h with ficin protease [.23 units/ml (25)].

⁴ Proportion of total nitrogen remaining soluble after addition to McDougall's buffer of trichloroacetic acid (TCA) to a final concentrate of 5% wt/vol (14). NPN = Nonprotein nitrogen.

⁵ Proportion of total nitrogen insoluble after treatment for 48 h with *Streptomyces griseus* protease [6.6 units/ml (20)].

⁶ Proportion of protein estimated to escape the rumen at observed degradation rate and a rate of passage (k_p) of .06 h^{−1}. Estimated escape, % = [$k_p/(k_p + k_d)$] × 100 (6).

for ash (2) and crude protein by the AOAC method (2) except that a copper catalyst² was used during digestion. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and ADIN were determined by the methods of Van Soest and coworkers (18, 26). Ration compositions are reported in Table 2.

Four hours after feeding on d 14 of each period, 5-ml blood samples were taken by venipuncture from the jugular vein of each cow, heparinized, and placed immediately on ice. Whole blood was stored at 2°C until analyzed the next day for urea (32). Plasma was prepared within 1 h of sampling and deproteinized with sulfosalicylic acid (11). Deproteinized plasma was stored (-20°C) until analyzed for plasma free amino acids using a Beckman 6300 Amino Acid Analyzer.³

Also on d 14 of each period, a rumen turnover study was conducted. Animals were pulse-dosed (via ruminal cannulae) just before feeding with 200 ml of a solution of the chromium chelate of ethylenediaminetetraacetic acid (Cr-EDTA) containing 20,000 µg/ml Cr (5). Samples of strained rumen fluid (SRF), taken from the ventral sac at 0 (just prior to dosing) at 1.5, 3, 4.5, 6, 8, and 10 h after dosing were prepared by straining through two layers of cheesecloth. Grab samples of 250 to 400 g of whole rumen contents (WRC) were taken from the ventral sac at 0, 3, 6, and 10 h. These samples of WRC were assumed to be representative of total rumen contents. The SRF was preserved with 1 ml 50% vol/vol sulfuric acid/50 ml SRF (16) and stored at -20°C until analyzed for Cr (13), ammonia (9), and volatile fatty acids (VFA). The VFA were determined with α-ethyl-n-butyrate as internal standard (W. C. Ellis, personal communication), using the SP-1200 column of Ottenstein and Bartley (23), which does not resolve isovalerate and 2-methyl butyrate. The WRC were dried (60°C, 72 h), ground (1 mm), and analyzed for DM [105°C (2)]. Total protein amino acids were determined in acid hydrolysates of WRC

(7). Liquid fractional turnover rates were determined as the slopes of linear regression of the natural log of Cr concentration over time. Liquid volume was computed by dividing the Cr dose by the antilog of the regression intercept. Rumen pools of DM and protein amino acids were then computed from the liquid volume and mean DM in each set of four WRC samples. Net protein amino acid pools (from undegraded SBM) were computed as the differences in rumen protein amino acid pools between cows fed diet U and S-1 or E-1 diets. It was assumed that undegraded feed protein (other than from SBM) plus microbial protein were equal on all three diets. Corn protein intake was greater on diet U; hence, both rumen pools of undegraded SBM protein were slightly underestimated. Undegraded SBM-N was computed by dividing net protein amino acids by the amino acid/N observed in acid-hydrolysates of each SBM (.0468 and .0478 mol total amino acids/g N for solvent and expeller SBM, respectively).

Mean data were analyzed as a 3 × 3 Latin square, replicated two times (30). Where significant F-values were detected due to diet (at least $P < .05$), mean separation was by least significant difference.

Trial 2

Twelve Holstein cows, including four with rumen cannulae, averaging 636 kg in weight, 31 (SD = 14) d in milk, 31.2 kg milk/d, and lactation number 3.4, were randomly assigned to two dietary treatments in a crossover experiment (12). Supplemental protein was provided by either solvent or expeller SBM (Table 2). Diet S-2 also contained added crude soybean oil to equalize fat content between rations. The balance of the diets was forage from second cutting alfalfa silage and corn silage, plus corn grain, minerals, and vitamin premix (Table 2). Alfalfa silage and corn silage contained 5.0 and 4.3% ADIN (% of total N), respectively. Diets were fed for 4 wk before switching (total 8 wk); the 1st wk of each period was considered as transitional, and production data were collected over the last 3 wk. Milk production was measured daily; milk was sampled at both milkings 2 d each week. Proportional composites were prepared and analyzed for fat by

² Kjeltabs, Tecator Inc., Herndon, VA 22070.

³ Spinco Division, Beckman Instruments, Palo Alto, CA 94304.

TABLE 2. Composition of diets.¹

Component	Trial 1		Trial 2		Trial 3				
	U	S-1	E-1	S-2	E-2	S-3a	E-3a	S-3b	E-3b
				(% dry matter)					
Alfalfa silage	20.6	20.7	28.1	28.3	28.2	28.0
Corn silage	56.7	56.5	56.50	34.8	34.9	29.0	29.2	29.1	28.9
Corn grain	38.0	19.3	19.3	31.0	31.0	35.6	37.3	31.8	35.7
Solvent soybean meal (SBM)	...	22.4	...	12.2	...	6.33	...	10.0	...
Expeller SBM	22.4	...	12.4	...	4.06	...	6.55
Urea	2.8929
Soybean oil50
Sodium sulfate	.35
Limestone	.86	.85	.85
Dicalcium phosphate	.86	.43	.43	.44	.44	.42	.42	.42	.42
Trace mineral salt	.44	.43	.43	.44	.44	.42	.42	.42	.42
Vitamin premix ²	.05	.05	.05	.04	.04	.04	.04	.04	.04
Chemical composition									
Crude protein	17.9	18.4	17.6	16.8	16.0	15.4	15.5	16.5	15.4
Soluble crude protein ³	64.6	36.2	20.9	40.6	33.1	41.3	45.3	40.3	38.0
SBM crude protein	0	10.9	10.1	5.38	5.38	3.09	1.84	4.91	2.97
Neutral detergent fiber	27.9	27.8	27.8	37.5	37.4	36.6	36.7	37.0	36.8
Acid detergent fiber	16.4	16.4	16.4	19.6	20.3	23.4	22.2	23.3	22.9
Ash	5.3	5.9	5.7	6.3	6.5	7.4	7.2	7.5	7.2

¹ Abbreviations: U = urea, S = solvent soybean meal, E = expeller soybean meal.

² Contained (per kg dry matter): 2.2 million IU vitamin A, 2.2 million IU vitamin D, and 220 IU vitamin E.

³ Proportion of total crude protein soluble in McDougall's buffer (14).

infrared analysis⁴ and protein by Kjeldahl N \times 6.38 (2). Milk was deproteinized by mixing with an equal volume of 25% TCA (wt/vol). The high speed (31,000 \times g, 15 min, 2°C) TCA supernatants were stored at -20°C until analyzed for lactose (31) and urea (32). Cows were weighed on 3 consecutive d at the start of the trial and at the end of each period.

Diets were fed ad libitum twice daily as total mixed rations (TMR). A weekly composite of each TMR and forage was collected from daily samples of about .5 kg and stored frozen. Feed refusals were determined every other day, and subsamples of refusals from each diet were composited and stored in the same manner. Forage content of as-fed rations was adjusted at the beginning of the study based on DM estimated at 60°C (48 h) and maintained at these ratios throughout. Actual proportion of dietary DM from each diet component was computed from DM determined as described in Trial 1. Analyses of composite samples of TMR for CP, ash, NDF, and ADF also were as detailed for Trial 1. Daily samples of TMR and feed refusals were analyzed for DM (60°C, 48 h), and DM intake (DMI) is reported on this basis. Composition of rations fed in Trial 2 are reported in Table 2. Four hours after feeding on d 27 of each period, 5-ml blood samples were taken from each cow by venipuncture from the tail artery or vein. Blood was heparinized and stored at 2°C until analyzed the next day for urea (32). Plasma was prepared from the balance of the blood and deproteinized and analyzed for free amino acids as described in Trial 1. On the same day, rumen samples were collected from the four cows fitted with ruminal cannulae. Samples of SRF were taken from the ventral sac at 0, 1, 2, 3, 4, and 6 h after the morning feeding. Samples were processed and analyzed for pH, ammonia, and VFA as detailed.

Mean data were analyzed using a crossover design, replicated six times, except for observations from the four ruminally cannulated cows, which were analyzed as a crossover study replicated two times (12). Where significant F-values were detected due to diet (at least $P < .05$), mean separation was by least sig-

nificant difference.

Trial 3

Twenty Holstein cows, including 4 with rumen cannulae, averaging 597 kg in weight, 47 (SD = 21) d in milk, 33.2 kg milk/d, and lactation 3.0, were randomly assigned to four dietary treatments in a replicated 4 \times 4 Latin square. The four diets differed in source of supplemental protein: 6.33% solvent SBM, 4.06% expeller SBM plus .29% urea, 10.04% solvent SBM, and 6.55% expeller SBM (diets S-3a, E-3a, S-3b, and E-3b, respectively; Table 2). The balance of the rations was forage from third cutting alfalfa silage and corn silage, plus corn grain, minerals, and vitamin premix (Table 2). Alfalfa silage and corn silage contained 2.9 and 4.6% ADIN (% of total N), respectively. Diets were fed for periods of 3 wk (total 12 wk); the 1st wk was considered transitional, and production data were collected over the last 2 wk of each period. Measurement of milk production and composition and body weights, as well as feeding, feed sampling, and analyses were as described in Trial 2.

Blood samples were taken on d 19 or 20 of each period and analyzed for urea and plasma free amino acids as described. Also on d 19 or 20, SRF samples were obtained from the cannulated cows; these were analyzed for pH and VFA as described for Trials 1 and 2.

Mean data were analyzed as a 4 \times 4 Latin square, replicated five times, except for observations from the four ruminally cannulated cows, which were analyzed as a single 4 \times 4 Latin square (30). Where significant F-values were detected due to diet (at least $P < .05$), mean separation was by least significant difference.

RESULTS AND DISCUSSION

Trial 1

Blood urea concentration was significantly less with feeding of E-1 than with either U or S-1 diets (Table 3). As expected, rumen ammonia was greatest with the U diet; however, ammonia concentration was also greater with S-1 than with the E-1 diet (Table 3). The branched-chain VFA, isobutyrate, isovalerate (3-methyl butyrate), and 2-methyl butyrate are produced in the rumen largely from deamination and decarboxylation of the branched-chain

⁴Wisconsin Dairy Herd Improvement Cooperative, 5301 Tokay Blvd., Madison 53711.

TABLE 3. Content of urea in milk and blood and concentrations of various metabolites in rumen fluid.¹

Component	Trial 1			SE	Trial 2			SE	Trial 3			SE
	U	S-1	E-1		S-2	E-2	S-3b		S-3a	E-3a	S-3b	
Milk urea, mM	4.59 ^a	3.62 ^b	.15	3.50 ^b	3.66 ^b	4.63 ^a	3.38 ^b	.13
Blood urea, mM	6.23 ^a	6.49 ^a	4.67 ^b	.39	4.93	4.58	.20	3.65 ^b	3.93 ^b	4.95 ^a	3.62 ^b	.12
Rumen fluid	6.58	6.43	6.46	.05	6.07	6.22	.11	6.35	6.29	6.37	6.40	.10
pH												
Ammonia-N, mg/dl	11.98 ^a	8.34 ^b	5.61 ^c	.86	12.57	13.11	.61	14.14 ^{ab}	18.58 ^a	17.08 ^a	9.76 ^b	1.57
Total VFA, mM	80.3 ^b	90.4 ^a	80.6 ^b	3.3	136.3	129.4	4.1	134.0	131.3	133.4	130.2	8.0
Acetate, molar %	66.1 ^a	62.4 ^b	63.5 ^b	.5	59.0	61.5	1.2	59.4	59.1	60.3	58.9	.6
Propionate, molar %	18.5 ^b	20.1 ^a	20.2 ^a	.4	24.9	22.1	1.4	22.6	22.6	21.6	23.3	.7
Butyrate, molar %	12.1 ^b	13.0 ^a	12.3 ^b	.2	11.5	12.1	.2	12.0	12.2	12.3	12.0	.2
Isobutyrate, mM	.50 ^c	.92 ^a	.69 ^b	.04	1.22	1.14	.07	2.52	2.44	2.38	2.39	.12
Isovalerate + 2-methyl butyrate, mM	1.29 ^b	1.84 ^a	1.58 ^{ab}	.09	2.35	2.24	.25	3.07	2.98	2.79	2.81	.16
Total BC-VFA, mM	1.80 ^c	2.76 ^a	2.26 ^b	.13	3.56	3.38	.31	5.59	5.42	5.17	5.20	.27
Valerate, mM	.88 ^b	1.39 ^a	1.04 ^b	.06	2.75	2.21	.40	2.49	2.49	2.42	2.31	.23

a, b, c Means in rows within each trial having different superscripts differ ($P < .05$).¹ Abbreviations: U = urea, S = solvent soybean meal, E = expeller soybean meal, VFA = volatile fatty acids, BC = branched chain. See Table 2 for composition of diets.

amino acids (1), whereas valerate is formed partly from catabolism of proline (19). Hence, concentrations of these VFA are related to ruminal protein degradation. Concentration of individual and total branched-chain VFA was less on the urea diet, reflecting its low content of preformed protein. Concentrations of isobutyrate, total branched VFA, and valerate were all less on the E-1 diet than the S-1; concentrations of isovalerate plus 2-methyl butyrate were intermediate (Table 3). The lower blood urea, rumen ammonia, and rumen branched-chain VFA indicate greater resistance to ruminal degradation of protein from expeller than solvent SBM. Comparable low concentrations of *Streptomyces griseus* insoluble N in both SBM (Table 1) indicate no extra heat damage in expeller SBM (20). Rumen total VFA and molar proportion of propionate and butyrate were greater, and molar proportion of acetate less, on the S-1 diet (Table 3). This may be related to the small, nonsignificant increase in feed intake with that diet (Table 4).

Results of the rumen turnover study are in Table 4. Although DMI and rumen DM pools were not influenced by diet, rumen pools of protein amino acids were greater ($P < .05$) on the S-1 and E-1 diets. Net rumen pool of undegraded SBM N was estimated by subtracting the protein amino acid pool on the U diet and dividing the result by the amino acid/N value of each SBM. Rate of total disappearance is the sum of the rate of passage (k_p) plus the rate of degradation (k_d). Dividing SBM N intake by the net rumen pool of undegraded SBM N yielded estimates of total ruminal disappearance rates ($k_p + k_d$) of .237 and .145 h^{-1} , for solvent and expeller SBM protein, respectively. Ruminal escape is given by the equation: $Escape = k_p / (k_p + k_d)$ (6). Therefore, ruminal escape of expeller SBM as a proportion of solvent SBM, may be computed from the ratio (19): $[(k_p / .145) / (k_p / .237)] \times 100$. The unknown passage rate (k_p) divides out and the relative ruminal escape becomes: $(.237 / .145) \times 100 = 164\%$, or 64% greater than solvent SBM. This trial was conducted with low producing or nonlactating cows with DMI lower than those of cows in early lactation. Although lower DMI should result in slower ruminal passage, relative ruminal degradability would still be valid, provided ruminal passage was similar on both diets.

TABLE 4. Dry matter intake and soybean meal nitrogen estimated as remaining undegraded in the rumen (Trial 1).

Item	Supplemental N source ¹			
	U	S-1	E-1	SE
Dry matter intake, kg/d	17.5	18.8	17.1	.98
Rumen dry matter pool, kg	12.3	13.0	13.3	.64
Rumen protein amino acid pool, mol	8.39 ^b	11.1 ^a	12.2 ^a	.67
Net protein amino acid pool, ² mol	...	2.69	3.80	...
Net protein N pool from SBM, ³ g (A)	...	57.5	79.5	...
SBM N intake, g/d (B)	...	327	276	...
Total SBM N disappearance rate, ⁴ h ⁻¹237	.145	...
Relative ruminal escape for SBM N, ⁵ %	...	100	164	...
Cr-EDTA ⁶ dilution rate, h ⁻¹	.117	.145	.117	.010

^{a,b} Means in rows having different superscripts differ ($P < .05$).

¹ Sources of supplemental nitrogen were urea (U), solvent soybean meal (S-1), or expeller soybean meal (E-1). See Table 2 for diet compositions.

² Difference between apparent protein amino acids when cows were fed diets S-1 and E-1 and diet U.

³ Computed by dividing net protein amino acid pool by amino acids per unit N in acid hydrolysates of solvent and expeller soybean meal (.0468 and .0478 mol/g N, respectively).

⁴ Total SBM-N disappearance rate (h⁻¹) = (B/A) × day/24 h. This is the sum of rates of degradation plus passage ($k_d + k_p$).

⁵ Relative ruminal escape for SBM-N computed assuming equal ruminal passage rate (k_p) for diets S-1 and E-1. Relative ruminal escape for expeller SBM, as a proportion of solvent SBM (19) = $[(k_p/.145)/(k_p/.237)] \times 100$.

⁶ Cr-EDTA = Chromium ethylenediaminetetraacetate.

Dilution rates of Cr-EDTA were nonsignificantly greater with diet S-1 (Table 4). However, the protein in SBM was largely insoluble (Table 1) and would be expected to move with the solid phase; hence, it would be inappropriate to estimate ruminal passage from dilution of the liquid marker, Cr-EDTA.

In estimating the relative escape of expeller SBM protein it was assumed that ruminal pools of microbial protein were equal across all three diets. It is possible that microbial protein synthesis was greater on the SBM diets, particularly on S-1. If the ruminal pool of microbial protein were greater with feeding of S-1 than that with E-1, the relative ruminal escape of expeller SBM would have been underestimated.

Plasma free amino acid concentrations, which were significantly affected by diet, are in Table 5. The diets containing SBM give rise to greater concentrations of individual and total branched-chain amino acids, branched-chain:glycine ratio, and total essential amino acids, all of which are consistent with greater supply of protein to the intestine (4). There was no apparent trend for greater concentrations of

these indicator amino acids between E-1 and S-1. However, lysine concentrations were less ($P < .05$) with E-1 than S-1. Intake of SBM protein was somewhat higher on the S-1 than E-1 diet (Table 4), and protein intake on all diets was less space in excess of requirement.

An interesting finding was that animals fed E-1 diet (Table 4), and protein intake on all diets was in excess of requirement.

positive compound that eluted between lysine and l-methyl histidine (Table 5). This postlysine peak was of very low concentration and similar in animals fed U or S-1. It is speculated that this compound was formed from lysine during heating in expeller SBM processing. The elevated postlysine peak and reduced plasma lysine imply reduced availability of lysine. This warrants further investigation.

Trial 2

Effects of feeding similar amounts of either S-2 or E-2 on DMI, weight change, and milk production are in Table 6. Body weight was

TABLE 5. Concentrations of free amino acids in blood plasma significantly affected by diets in Trials 1, 2, or 3.¹

Component	Trial 1			SE	Trial 2			SE	Trial 3			SE
	U	S-1	E-1		S-2	E-2	S-3		S-3a	E-3a	S-3b	
(nmol/ml plasma)												
Alanine	158	190	176	11	233	212	11	246 ^a	239 ^{a,b}	214 ^b	244 ^a	8
Citrulline	60 ^b	62 ^{a,b}	70 ^a	3	61	66	3	70	70	71	69	2
α-Amino butyrate	10 ^b	14 ^a	11 ^{a,b}	.8	9	10	.8	13	15	14	12	.8
Valine	143 ^b	272 ^a	260 ^a	22	218	248	12	210 ^{a,b}	206 ^b	222 ^{a,b}	226 ^a	6
Methionine	20 ^a	20 ^a	15 ^b	1	22	20	2	23	22	21	22	1
Isoleucine	72 ^b	123 ^a	114 ^a	10	101 ^b	117 ^a	7	97	97	97	103	3
Leucine	120 ^b	175 ^a	169 ^a	16	149 ^b	177 ^a	9	145 ^{a,b}	147 ^{a,b}	136 ^b	156 ^a	5
Ornithine	36 ^b	56 ^a	55 ^a	5	50	49	4	43	42	43	44	2
Lysine	55 ^b	83 ^a	61 ^b	5	82	75	6	70	70	75	73	3
Postlysine peak ²	2 ^b	4 ^b	12 ^a	1	3 ^b	16 ^a	1	3 ^c	9 ^b	4 ^c	10 ^a	.4
Histidine	49	56	57	3	54	55	3	51 ^b	55 ^{a,b}	50 ^b	61 ^a	7
Arginine	65 ^b	78 ^a	68 ^b	3	73	74	5	66	67	69	71	3
Sulfur amino acids	35 ^a	34 ^a	28 ^b	1	30	28	2	36	34	32	35	1
Branched-chain amino acids ³	336 ^b	571 ^a	544 ^a	48	468 ^b	542 ^a	28	453	450	455	484	13
Branched-chain amino acids/glycine	1.46 ^b	2.55 ^a	2.21 ^a	.21	1.04	1.13	.07	1.20 ^b	1.21 ^b	1.38 ^a	1.26 ^b	.04
Essential amino acid	680 ^b	994 ^a	909 ^a	70	885	946	49	860	848	851	917	25
Nonessential	1321	1345	1323	64	1625	1656	77	1545	1563	1455	1546	36

a,b,c Means in rows within each trial having different superscripts differ ($P<.05$).

¹ Abbreviations: U = urea, S = solvent soybean meal, E = expeller soybean meal. See Table 2 for diet composition.

² Relative concentration units based on the internal standard, S-2-aminoethyl cysteine.

³ Sum of concentrations of valine, isoleucine, plus leucine.

TABLE 6. Dry matter intake, weight change, and production of milk and milk components (Trials 2 and 3).

Item	Trial 2			Trial 3				SE
	S-2	E-2	SE	S-3a	E-3a	S-3b	E-3b	
Dry matter intake, kg/d	20.4	19.8	.4	22.8 ^a	22.4 ^a	21.2 ^b	22.4 ^a	.4
Weight change, kg/d	.06	-.10	.86	.44	.21	.25	.55	.16
Milk, kg/d	35.1	35.4	.5	34.4 ^{ab}	32.6 ^c	33.3 ^{bc}	35.2 ^a	.5
Milk/dry matter intake	1.73 ^b	1.79 ^a	.02	1.51 ^b	1.45 ^b	1.58 ^a	1.57 ^a	.02
4% FCM, kg/d	30.2	30.4	.4	31.7 ^{ab}	30.6 ^b	31.3 ^{ab}	32.5 ^a	.5
Fat, %	3.04	3.02	.07	3.50	3.61	3.52	3.52	.06
Fat, kg/d	1.07	1.08	.02	1.20	1.17	1.17	1.23	.02
Protein, %	2.98 ^a	2.84 ^b	.03	3.09	3.04	3.06	3.04	.02
Protein, kg/d	1.04	1.00	.02	1.06 ^a	.98 ^b	1.01 ^b	1.07 ^a	.02
Lactose, %	4.99	4.95	.03	4.97	4.97	4.94	4.99	.03
Lactose, kg/d	1.75	1.75	.03	1.70 ^{ab}	1.62 ^c	1.64 ^{bc}	1.75 ^a	.03

^{a,b,c}Means in rows within each trial having different superscripts differ ($P < .05$).

¹ Abbreviations: S = Solvent soybean meal, E = expeller soybean meal, FCM = fat-corrected milk. See Table 2 for diet compositions.

essentially maintained on both diets. Generally, production of milk and milk components was not influenced by source of SBM with two exceptions: production efficiency (milk/DMI) was increased about 4% ($P < .05$) and milk protein was reduced from 2.98 to 2.84% ($P < .05$) with the feeding of E-2. It was surprising that there was no improvement in milk production despite convincing evidence from N solubility and in vitro analyses (Table 1), and rumen turnover data (Table 4) that expeller SBM protein is substantially more resistant to ruminal degradation.

Concentration of urea in milk, but not blood, was less ($P < .05$) with feeding of E-2 (Table 3). Ruminal ammonia was not altered by diet. Although there were trends for reduced branched-chain VFA and valerate concentrations with feeding of E-2 versus S-2, the influence of diet on VFA concentrations and molar proportions in the rumen was non-significant (Table 3).

Although most amino acids were not effected by diet, concentrations of isoleucine, leucine, and total branched-chain amino acids (valine, isoleucine, and leucine) were elevated with the E-2 diet (Table 5). This has been associated with increased protein supply to the small intestine (4). Lack of production response suggests that intestinal protein supply was not limiting milk secretion in this trial. The possibility of reduced lysine availability was dis-

cussed earlier. Lactating cows consistently have yielded increased production with abomasal protein infusions (10) and supplemental protein in the diet (27). However, other studies have resulted in only small (17) or no advantage (21) when using resistant proteins to make iso-nitrogenous replacements of conventional protein sources.

As in Trial 1, there was substantial elevation of a postlysine compound with the feeding of E-2 (Table 5).

Trial 3

Diet S-3b was formulated to have the same crude protein content as the diets in Trial 2; the other three diets provided about 60% as much supplemental crude protein (Table 2). Feed intake, weight gain, and milk production results from this trial are summarized in Table 6. There was reduced DMI with diet S-3b. Weight gain was not influenced by diet. Generally, production of milk and milk components was greatest with diet E-3b, intermediate on diet S-3a, and least on diets E-3a and S-3b (Table 6). A notable exception to this trend was production efficiency (milk/DMI), which was approximately equal and greater on diets S-3b and E-3b and lower on diets S-3a and E-3a. Concentrations of milk fat, protein, and lactose were similar on all diets.

Improvement in performance with increasing protein supplementation above that fed in diet

E-3a indicated that protein supply limited production. There was a trend for greater production with diet E-3b when compared with equal supplementation from diet S-3a (Table 6). Unexpectedly poorer performance was obtained with diet S-3b (Table 6). This may be partly explained by the reduced DMI. However, diets S-3b and E-3b gave similarly high efficiencies of production (Table 6), even though diet E-3b provided only 60% as much supplemental protein (Table 2). Efficiency of production on diets S-3a and E-3a were lower and not different ($P>.10$) from each other. Satter (28) has suggested that the most effective strategy for feeding resistant proteins may be to replace a larger quantity of conventional protein with less of the resistant protein. Economic advantage would then be due to reduced feed costs rather than increased production.

Milk urea concentrations tended to mirror those in blood (22); milk urea was greatest on diet S-3b (Table 3). As expected, rumen ammonia was greatest on the urea-containing diet (E-3a) and diet S-3b. However, ammonia concentrations were less on E-3b than S-3a at equal dietary protein, reflecting the greater resistance of expeller SBM to degradation. Performance was not comparable between diets S-3a and E-3a (Table 6), although they had the same ratio of solvent SBM to expeller SBM as diets S-3b and E-3b (Table 2). This suggests an adverse effect due to feeding urea. However, rumen ammonia, although greatest on diet E-3a, was not in the concentration ranges where suppression of animal performance has been reported (3). It is interesting that milk and blood urea did not reflect the high ruminal ammonia concentrations observed on diet E-3a. No significant trends were detected for ruminal pH or VFA in this experiment.

Plasma free amino acid concentrations from this trial were largely unaffected by diet (Table 5). There were no clear trends in branched-chain amino acids, and only small differences were observed in plasma alanine and histidine. This may be expected because increased milk production would serve as a "sink" for additional absorbed amino acids. However, feeding diet S-3b (Table 2) resulted in an elevation in the branched-chain amino acid: glycine ratio, which has been correlated to improved protein status (4). As in the other

two feeding trials, feeding of E-3a or E-3b yielded increased plasma concentrations of the postlysine compound. Its concentration was greatest on diet E-3b, intermediate on diet E-3a, and least on diets S-3a and S-3b (Table 5).

CONCLUSIONS

Protein solubility and in vitro and in vivo protein degradability estimates indicated that expeller SBM, which was heated to a maximum of 163°C during processing, provided about 65% more undegraded protein than solvent SBM. When fed in amounts equal to solvent SBM in diets with 16 to 17% CP, there was no advantage in increased milk production. However, when fed in the ration at lower amounts, expeller SBM gave production comparable with greater quantities of solvent SBM.

ACKNOWLEDGMENTS

The author gratefully acknowledges Len Strozinski and his coworkers for care and feeding of the cows. The excellent technical assistance of Mike Meyer, Debby O'Brien, and Heidi Mier is greatly appreciated. Plasma amino acid analyses were conducted by Brad Ricker.

REFERENCES

- 1 Allison, M. J. 1970. Nitrogen metabolism of ruminal micro-organisms. Pages 456 to 473 in *Physiology of digestion and metabolism in the ruminant*. A. T. Phillipson, ed. Oriel Press, Newcastle upon Tyne, England.
- 2 Association of Official Analytical Chemists. 1980. *Official methods of analysis*. 13th ed. Assoc. Offic. Anal. Chem. Washington, DC.
- 3 Bartley, E. E., A. D. Davidovich, G. W. Barr, G. W. Griffel, A. D. Dayton, C. W. Deyoe, and R. M. Bechtel. 1976. Ammonia toxicity in cattle. I. Rumen and blood changes associated with toxicity and treatment methods. *J. Anim. Sci.* 43:835.
- 4 Bergen, W. G. 1979. Free amino acids in blood of ruminants — physiological and nutritional regulation. *J. Anim. Sci.* 49:1577.
- 5 Binnerts, W. T., A. Th. Van't Klooster, and A. M. Frens. 1968. Soluble chromium indicator measured by atomic absorption in digestion experiments. *Vet. Rec.* 82:470.
- 6 Broderick, G. A. 1978. In vitro procedures for estimating rates of ruminal protein degradation and proportions of protein escaping the rumen undegraded. *J. Nutr.* 108:181.
- 7 Broderick, G. A. 1984. In vitro determination of rates of ruminal protein degradation. *Can. J. Anim. Sci.* 64 (Suppl.):31.

- 8 Broderick, G. A., and W. M. Craig. 1980. Effect of heat treatment on ruminal degradation and escape, and intestinal digestibility of cottonseed meal protein. *J. Nutr.* 110:2381.
- 9 Broderick, G. A., and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. *J. Dairy Sci.* 63:64.
- 10 Broderick, G. A., T. Kowalczyk, and L. D. Satter. 1974. Milk production response to supplementation with encapsulated methionine per os or casein per abomasum. *J. Dairy Sci.* 53:1714.
- 11 Broderick, G. A., L. D. Satter, and A. E. Harper. 1974. Use of plasma amino acid concentration to identify limiting amino acids for milk production. *J. Dairy Sci.* 57:1015.
- 12 Cochran, W. G., and G. M. Cox. 1957. Experimental designs. John Wiley and Sons, New York, NY.
- 13 Combs, D. K. 1985. An evaluation of markers and techniques used to measure nutrient digestion in ruminants. Ph.D. Thesis, Univ. Wisconsin, Madison.
- 14 Craig, W. M., and G. A. Broderick. 1981. Comparison of nitrogen solubility in three solvents to in vitro protein degradation of heat-treated cottonseed meal. *J. Dairy Sci.* 64:769.
- 15 Dewar, W. A., and P. McDonald. 1961. Determination of dry matter in silage by distillation with toluene. *J. Sci. Food Agric.* 12:790.
- 16 Erwin, E. S., G. J. Marco, and E. M. Emery. 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. *J. Dairy Sci.* 44:1768.
- 17 Folman, Y., H. Newmark, M. Kaim, and W. Kaufmann. 1981. Performance, rumen and blood metabolites in high-yielding cows fed varying protein percents and protected soybean. *J. Dairy Sci.* 64:759.
- 18 Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analysis (apparatus, reagents, procedures, and some applications). US Dep. Agric., Agric. Handbook No. 379, US Dep. Agric. Washington, DC.
- 19 Hungate, R. E. 1966. The rumen and its microbes. Academic Press, New York, NY.
- 20 Krishnamoorthy, U., C. J. Sniffen, M. D. Stern, and P. J. Van Soest. 1983. Evaluation of a mathematical model of rumen digestion and an in vitro simulation of rumen proteolysis to estimate the rumen-undegraded nitrogen content of feedstuffs. *Br. J. Nutr.* 50:555.
- 21 Mielke, C. D., and D. J. Schingoethe. 1981. Heat-treated soybeans for lactating cows. *J. Dairy Sci.* 64:1579.
- 22 Oltner, R., and H. Wiktorsson. 1983. Urea concentrations in milk and blood as influenced by feeding varying amounts of protein and energy to dairy cows. *Livest. Prod. Sci.* 10:457.
- 23 Ottenstein, D. M., and D. A. Bartley. 1971. Separation of free acids C₂-C₅ in dilute aqueous solution column technology. *J. Chromatogr. Sci.* 9:673.
- 24 Pena-Castellanos, F. 1983. Heat treatment of protein supplements for improving protein utilization by dairy cows. Ph.D. Thesis, Univ. Wisconsin, Madison.
- 25 Poos-Floyd, M., T. Klopfenstein, and R. A. Britton. 1985. Evaluation of laboratory techniques for predicting ruminal protein degradation. *J. Dairy Sci.* 68:829.
- 26 Robertson, J. B., and P. J. Van Soest. 1977. Dietary fiber estimation in concentrate feedstuffs. *J. Anim. Sci.* 45 (Suppl. 1):254. (Abstr.)
- 27 Roffler, R. E., J. E. Wray, and L. D. Satter. 1986. Production responses in early lactation to additions of soybean meal to diets containing predominantly corn silage. *J. Dairy Sci.* 69:1055.
- 28 Satter, L. D. 1986. Protein supply from undegraded dietary protein. *J. Dairy Sci.* 69: (in press).
- 29 Sherrod, L. B., and A. D. Tillman. 1964. Further studies on the effects of different processing temperatures on the utilization of solvent-extracted cottonseed protein by sheep. *J. Anim. Sci.* 23:510.
- 30 Steel, R.G.D., and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., New York, NY.
- 31 Technicon. 1973. Lactose in milk. Technicon Industrial Method No. 120-71A. Technicon Ind. Syst., Tarrytown, NY.
- 32 Technicon. 1977. Urea nitrogen. Technicon Industrial Method No. 339-01. Technicon Ind. Syst., Tarrytown, NY.
- 33 Wohlt, J. E., C. J. Sniffen, and W. H. Hoover. 1973. Measurement of protein solubility in common feedstuffs. *J. Dairy Sci.* 56:1052.